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Dynamic Response of Single Neuronal Units to Frequency-Modulated Auditory Stimuli in the Unanaesthetized Animal.

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ABSTRACT

The object of the research is to study the dynamic, as opposed to the steady state, properties of the auditory pathway. To this end, and specifically, the response of single units in the auditory cortex of the unanaesthetised unrestrained cat have been recorded under various types of acoustic stimulation.

In order to do this the first requirement was to design and build a generator which would provide audio-frequency stimuli capable of being modulated in respect of both frequency and amplitude in any desired manner. A major problem was to obtain adequate frequency modulation free from any significant accompanying amplitude modulation. A generator has been designed which will provide frequency modulation up to $\pm$ 6% of the centre frequency with <1% variation in amplitude, and provide also independent amplitude modulation up to a depth of 80%. The centre frequency can be varied over the range of 300 c/s to 25 kc/s. The modulation envelope can be of any waveform which can be generated as a voltage, and the two types of modulation can be independently varied, and mixed in any required relationship.

Records were made by means of micro-electrodes operated by a micro-manipulator attached to the cat's skull. The cat was placed in a spacious cage situated in the anechoic test-room. The stimuli and responses were recorded on magnetic tape and subsequently transferred to film. Records were obtained from 91 units in various parts of the auditory cortex. Just under 80% of these units would respond to some form of acoustic stimulation.

In cases where they would respond to tones the characteristic frequencies of the units were determined and the spatial distribution of their frequencies on the cortical surface mapped. About three-quarters of the acoustically sensitive units were so responsive, the other one-quarter being responsive only to changing and transient sounds.

One fifth of all the units were unresponsive to acoustic stimuli under the experimental conditions. Three of these units responded to visual stimuli, while the rest did not appear to respond to any sensory stimulation. The relation between such failure to respond, and the condition of the cortex is discussed. Considerable long term variations in sensitivity following stimulation were observed in some units.

Both units which responded to steady tones and units which would respond only to changing stimuli were subjected to frequency-modulated stimuli. In the former the phase relationships between the stimuli and the responses were those which would be predicted from threshold/frequency response curves. Of the few units which would respond only to 'gliding' tones, one, when subjected to frequency-modulated stimuli showed a phase-relationship strongly dependent on the modulation frequency. This dependence was not explicable in terms of transmission time in the system.

INTRODUCTION

The general behaviour of single units at various neural levels in the auditory system of mammals under anaesthesia is known from the studies of many workers, e.g. Galambos & Davis (1943), Thurlow, Gross, Kemp & Lowy (1951), Galambos (1952), Hilali & Whitfield (1953), Tasaki (1954), Tasaki & Davis (1955), Sumi, Katsuki & Uchiyama (1956), Erulkar, Rose & Davies (1956), and many more. Recently Stopp & Whitfield (1961) have obtained similar data for single units in birds. From a comparison of such data and of the known anatomical structures [e.g. Békésy (1951); Cajal (1909); Lorente de No (1933)], Allanson & Whitfield (1955) developed a hypothesis concerning the mechanism of pattern transformation in the cochlear nucleus and these ideas have been developed into a general theory of information carriage within the auditory system (Whitfield, 1957; 1959). The data from which the theory was originally derived were essentially measurements of the steady-state response of the system, and it is probable that neural elements have important dynamic properties not deducible from such measurements. It is known, for example, that there are units in the cat auditory system which will respond when the audio-oscillator control is turned steadily through a band of frequency, but will not respond when any specific frequency within that band is presented. Analogous effects in the visual system have been reported by Hubel (1959). The theory thus proposed is capable of handling, and has been tentatively extended to, signals such as those of speech, but more precise data on the temporal characteristics of the system are needed and the present report outlines the beginning of an attempt to obtain such data in a form which can be integrated with the general body of steady-state results. The responses of auditory units to clicks have frequently been determined [e.g. Galambos, Rose, Bromiley & Hughes (1952)] and this stimulus is in fact commonly used as a 'search-device' when locating units. Nevertheless these latter stimuli are not very useful in determining dynamic response since they have a broad and very indeterminate spectrum. It is virtually impossible to produce a 'square' acoustic pulse, and the common practice of applying a square electrical pulse to a transducer merely gives rise to a sound wave whose characteristics vary with the ringing property of the individual transducer concerned. Sounds which are changing in frequency and/or intensity in some continuous and regular manner provide stimuli whose results are more readily interpretable [Whitfield (1957)], and such stimuli have been used in these studies.

There is evidence [e.g. Woolsey & Walzl (1942); Rose, Galambos & Hughes (1959)] of a reasonably orderly projection of fibres from the cochlea through the various levels of the nervous system to the cortex. Thus at each level a stimulus of a particular frequency would, in the light of our theory already referred to, be expected to produce an anatomically defined area or band of active elements, which would change in position with frequency, or in extent with intensity.

If the static conditions can be extended to cover the dynamic situation, stimuli continuously varying in frequency or intensity would produce active regions, changing progressively in position and extent at the higher levels of the nervous system. Relatively little information is available concerning the behaviour of the auditory system to such stimuli. Experiments by Whitfield (1957), recording the evoked surface waves of the primary auditory cortex of the unanaesthetized cat with frequency-modulated tones, appear to support the theory at the cortical level. A rather sharply limited area of cortex appeared to be 'activated' by a given frequency, and this 'active area' moved across the cortex as the frequency changed.

What evidence there is at the neural unit level, however, suggests that the response of the nervous system to changing stimuli, may be somewhat different to the steady-state response.

Although there have been many studies of unit responses especially at lower levels of the auditory system and in unanaesthetised animals, only two appear to have been carried out on the cortex of the unanaesthetised and unrestrained preparation, those by Galambos (1960), and Katsuki (1959b) in the cat, and by Katsuki (1960) in the monkey. These studies have been conducted mainly using steady tones, tone pips, and clicks as stimuli. Katsuki also used voice sounds and beating tones, and Galambos gliding tones, white noise and 'odd' sounds. Such non-steady stimuli are too complex to allow of easy interpretation of the results in terms of theories of hearing. In the present study tones varying in a controlled manner in frequency and intensity are being used to investigate the 'dynamic' responses of cortical auditory units in 'normal' animals. The most easily-handled stimuli of this kind are tones where frequency and amplitude can be independently modulated by a mathematically well-defined (e.g. sinusoidal) waveform.

Much initial time in this project has been spent in establishing the technique of unit recording from unanaesthetised, unrestrained cats after the manner of Hubel (1957, 1959), producing the tone generators and appropriate environment for these studies, and in determining the general behaviour of the neural units obtained. In particular it has been necessary to obtain a method of producing frequency modulation which was entirely free from amplitude modulation, since the auditory system is relatively sensitive to loudness changes, and to ensure that the free field stimulation situation was free from standing waves, which would otherwise re-introduce spurious intensity changes when the frequency was changed. These problems are referred to again in the appropriate sections.

METHODS

The electrical responses of the neural units are recorded from insulated tungsten micro-electrodes, advanced into the cortex by a micromanipulator. The micromanipulator is located on a plastic 'plug' previously implanted into the cat's skull under anaesthesia.

Microelectrodes

These are made from 125 micra diameter tungsten wire, by electrolysis of the terminal 5 mm to a smooth taper. A modification of the method of Hubel (1957) is employed The wire is electrolysed against a carbon rod in a saturated solution of potassium nitrite with a potential of 5 volts A.C., Hubel's published method produces very abrupt tapers; to prevent this, the wire is moved up and down in the solution through a distance of 5 mm, by a motor-driven syringe system. By this means, good electrodes with final tip diameter of 1 micron or less are consistently produced with little experience.

The electrodes are washed in distilled water and detergent, and dried with acetone. They are then coated with an insulating plastic to within 5-25 micra of the tip. Hubel's (1957) method involves repeated dipping of the electrode into a vinyl lacquer, until all but the very tip is coated with polymer. We have found polystyrene in a benzene or chloroform solution very satisfactory, and have devised a simple method of coating the electrode. The electrode is raised slowly, tip uppermost, from a solution of syrupy consistency, whilst the solvent is rapidly evaporated from the coating by a small air jet. By this means reliably insulated electrodes are consistently and quickly produced with little practice. The electrodes are dried under a dust cover for 24 hours, and do not need baking. Polystyrene binds well to the metal, is highly insulating, and withstands immersion in saline for very long periods. Being strong but pliable, the insulation remains intact even though the tip be bent.

When dried, the electrodes are tested for continuity and extent of the insulation by watching for gas bubbles rising from the electrode immersed in saline, with a P.D. of 6V between the electrode (negative) and a carbon rod. More precise testing of the insulation, and impedance measurements, are effected by observing the voltage drop across a 10 megohm resistor, in series with the electrode dipped in saline. A 10 mV square wave is applied across resistor and saline solution, and measurements at the rising edge and plateau of the square wave give an indication of the high frequency impedance and D.C. resistance, respectively. The former reflects the ability of the very thin insulation near the tip to conduct the high frequency neuronal discharges, a property which extends the 'field' of recording from that of the bare tip alone. The high frequency impedance ranges from 3-25 megohms, and the D.C. resistance from ten to several hundred megohms.

Plug and Micromanipulator

The plastic plug is implanted into the skull under nembutal anaesthesia at least 24 hours before the first recording session. With aseptic precautions, the skull is exposed and an 8 mm diameter hole trephined through the bone at a point over the primary auditory cortex determined stereotactically, or by reference to the coronal suture. It is difficult to implant the plug over the posterior and lower end of the primary area in most cats because of the close proximity of the pinna and subsequent irritation to the animal. The hole is tapped with a bottoming tap, the dura overlying the cortex carefully excised, and the plug screwed into the tapped hole, where it forms a very tight fit, flush with the inner surface of the skull. The skin is sutured around the plug, and a stainless steel wire 'ground' electrode sutured to the scalp and brought out through a puncture wound in the upper part of the neck. It is bent upon itself so as to lie in the fur of the lower aspect of the head, and not interfere with the animal's toilet procedures, while remaining accessible to connection with a 'crocodile clip'. In between recording sessions the hollow centre of the plug is closed with a plastic core, and the unit passes apparently unnoticed by the animal. Penicillin is injected intramuscularly at the end of the procedure, and the cat is usually fully conscious six to twelve hours after operation. It is our practice to record nearly every day for two weeks after implantation, transfer the plug to the other side of the skull and record for a further two weeks. This procedure is necessary on account of the dura regrowing to a thick mass within the two weeks. Recordings have, however, been obtained through the thickened dura after one month.

Before recording from the animal, an aluminium adaptor is screwed onto the plug after removing the core, the cavity so formed filled with liquid paraffin warmed to 37°C, and the small micromanipulator holding the microelectrode clamped in place. Thus an oil-tight chamber, containing both the microelectrode and an indifferent electrode which rests lightly on the cortical surface, is created above the cortex. The original adaptor, after the pattern of Hubel has been modified to produce more effective sealing, and to allow the micromanipulator fitting into the adaptor to be rotated without breaking the seal. Being eccentrically placed in the adaptor, the microelectrode holder can be rotated in a 4 mm diameter circle over the cortex.

The microelectrode is inserted into a small tube attached to the piston of the micromanipulator. This piston moves in a cylinder filled with liquid paraffin connected via two flexible polythene tubes to syringes driven by a micrometer. The latter are located on the cage in which the animal lies during recording, and allow the microelectrode to be driven in 10-20 micra steps through the cortex in either direction whilst the animal is completely unrestrained. Although the distance between depths of the electrode can be estimated accurately from the micrometer readings, the absolute depth in most cases can only be roughly estimated owing to the unknown distance between the cortex and the electrode tip when the micromanipulator is placed in position. Often, contact of the electrode with the cortex is signalled by a transient displacement of the baseline, and the sudden appearance of a slow wave in response to clicks; this allows of more accurate estimation of depth. In no case was the electrode inserted into the cortex deeper than 5 mm.

Signals are led from the manipulator by a short length of anti-static screened cable to the input of the cathode-follower mounted on the recording cage. The screen is cathode-connected to minimise capacitative loss of signal.

Potential changes at the cortical surface are recorded from the indifferent electrode with an 'Ediswan' pen recorder.

The cat is free to move about in the cage during recording, and may turn around, and wash, with minimal interference to the recording. Without sedation, the cats have regularly lain or sat quietly for periods of up to eight hours.

Sound-proof Room

The open-mesh recording cage is suspended from the ceiling of the 'sound-proof' room. This room is an 8 ft. cube, constructed of 2" compressed straw sheet lined with 2" of glass wool on the inside surface. It is separated by a 1" air gap from the walls, floor, and ceiling of the room containing it. The front wall is separated from the recording ante-room by a 4" steel and glass wool partition through which access is obtained by double doors.

The floor is also covered by absorbent material and pyramids of similar material are placed at suitable points within the volume of the room to minimise standing waves. The optimal distribution and positioning of these pyramids is determined empirically by means of local sound pressure measurements. The problem is, of course, much greater at low frequencies and to eliminate all possibility of spurious results from intensity differences so produced, stimuli delivered to the experimental animals are confined to the frequency band above 300 c/s.

The attenuation afforded by the room to sounds outside, rises from 50 db at 300 c/s to over 80 db at 5 kc/s and rises still further above that frequency. This is very adequate for work in the cat's frequency range.

Sound-producing and Recording apparatus

Tones are generated by a Solartron Wide Range Oscillator or by the A.M.-F.M. Generator, described below, fed through a Leak 10 watt amplifier to a 10" Golden Wharfdale Loudspeaker mounted on a glass wool covered baffle placed one to two feet from the cat. Measurement of sound pressure levels inside the cage over a 200 c/s - 10 kc/s range shows a reasonably flat response with a maximum at 4-4.8 kc/s, tailing off shortly before 10 kc/s is reached. The sound system surprisingly produces sufficient intensities at frequencies in the 20-40 kc/s range to stimulate well many neural units responding at these frequencies.

The signals from the cathode-follower are fed via a Tektronix Type 122 Preamplifier to a display oscilloscope, also to an amplifier and speaker for aural monitoring of the unit discharge. Simultaneously with the output of the oscillators, the unit spike voltages are recorded onto magnetic tape by means of a very low impedance cathode-follower terminated amplifier (Coaton & Whitfield, 1954) feeding one recording head of a conventional tape recorder deck. The recorded unit responses, with the stimulating tones, can be replayed at leisure and filmed by a camera attached to the oscilloscope.

The effective noise level is 30 µV peak-to-peak with the cathode-follower input shorted, and 50 - 100 µV when terminated by the microelectrode dipping in saline solution.

The A.M.-F.M. Generator delivers tones between 300 c/s and 25 kc/s. These can be modulated in frequency by up to 5% of the centre value and can be amplitude-modulated up to a depth of 80%. The modulation is derived from a very-low-frequency oscillator giving a sinusoidal voltage at 3-30 c/s. Amplitude- and frequency-modulation can be combined in any desired way in respect both of range and relative phase.

A major problem was that of producing a frequency-modulated waveform of the required characteristics without an accompanying amplitude variation. The usual methods of amplitude stabilization did not give the required degree of stability in the range of modulation frequencies desired. Eventually a design was worked out which produced a waveform having less than 1% amplitude-modulation at the full depth of frequency-modulation. This degree of variation is below the limit of detectability by the ear.

The modulation waveform is also used to amplitude-modulate a 5 kc/s oscillator. The resultant output, whose envelope is in exact phase with the frequency modulation of the sound reaching the cat, is recorded simultaneously on the magnetic tape. This 'monitoring' signal allows the relation between the phase of firing of the neural units and that of the stimulus modulation envelope to be studied.

Both the Solartron Oscillator and the A.M.-F.M. Generator have adjustable attenuators with a range of 60 db. An additional 20db pad, built into the Leak amplifier, allows signals with a total intensity range of 80 db to be delivered to the animal. The maximum intensity possible with the equipment at 1,000 c/s was 100 db above the standard reference level of .0002 dynes/cm^2, measured inside the recording cage.

Electrode track marking

Electrolytic lesions are made in the cortex at the tip of the electrode immediately following recording, by passing a current of 5 μA through the electrode, with electrode negative. An apparatus has been made which will provide this current while allowing variations in the electrode resistance from zero to 100 megohms, giving at the same time an indication of that resistance.

Perfusion

At the end of the experiment, or within twelve hours of making lesions, the animal is anaesthetised, and the head perfused with 2 litres of normal saline and 10% formal-saline, via an internal carotid artery. The paraffin-embedded tissue is stained with a modified Kluver and Barrera's stain. The electrode tracks can be identified, varying from small blood-filled spaces to lines of 'round cells' extending into the cortex from the surface.

RESULTS

General

Ten plugs have been successfully implanted into six cats. One cat of the series died one week after operation (three recording days) from an unrelated condition. The cats have shown no ill-effects of implantation, and have been kept as long as several months before perfusion. Towards the end of such long periods, the implant has often become loose fitting, presumably due to foreign-body reaction of the bone. Histology of the brains shows a greatly thickened fibrous dura, but no evidence of infection has been seen.

Ninety-six 'units' have been studied in the six cats, 88 from the last four animals. Of the 96, the activity of 52 has been recorded, the majority of the remainder being units not responding to any sound, or to clicks only.

Fifty-six percent of the 'spikes' recorded were initially negative. Their mean height was about 600 µV. The remainder of the spikes were initially positive, of high voltage (mean 1.5 mV) and well resolved, and could be held for relatively long periods of time without signs of damage. On occasion two units, both showing initially positive spikes, were recorded simultaneously. This strongly suggests that the positive spikes are recorded extracellularly; the ability to record them for long periods lends support to this conclusion. Similar criteria were used by Erulkar, Rose & Davies (1956).

Cortical Depression

About 20% of the units studied could not be excited by any available stimulus, auditory, visual or tactile. It was evident that the great majority were obtained when the cortex was relatively inactive. This was indicated both by the absence of a gross response to auditory stimulation, and the fact that few other units which would respond to sound stimuli were seen at these times. In the initial experiments many such non-auditory units were seen and in fact no responsive units were obtained. Only after careful attention was given to ensuring that the liquid paraffin filling the plug chamber was at the correct cortical temperature were auditory units obtained, and the incidence of non-auditory units correspondingly reduced. Even then we observed little or no unit activity, especially auditory, in the first hour after fitting the micromanipulator to the plug. 70% of the non-auditory units studied were obtained in this period, compared with only 17% of the auditory units. It would therefore appear that even with great attention to temperature the cortex is sufficiently disturbed by the entrance of the liquid paraffin and indifferent electrode into the plug cavity for it to be depressed to a greater or lesser extent. This depression may last for a considerable period of time; on some occasions no unit, or at best no auditory unit, activity was observed for the whole of the recording session of several hours. An important factor may be the localised pressure changes occurringduring the removal of the core of the plug and the fitting of the micromanipulator. The latter involves a slight piston-like action of the manipulator on the liquid paraffin, as it is inserted into the aluminium adaptor on the plug.

No significant changes in the electrocorticogram were seen during these periods of depression of unit activity; at times the surface waves evoked by clicks appeared larger than usual.

Five 'units', found in otherwise inactive regions were not included in the classification above. They could not be stimulated, were more than 1-2 msec in duration, frequently diphasic, and fired regularly in time. Their spike heights were greater than 1 mV, and could be altered by moving the electrodes relatively large distances, of the order of hundreds of micra. 'Normal' units can be damaged or 'lost' by movements of the order of tens of micra.

General Behaviour of Units

The majority of the units displayed spontaneous firing, the discharge occurring in 'bursts', a phenomenon observed also in the visual cortex by Hubel (1959). Many units were completely silent, or discharged only one spike every few seconds, whilst others showed a high 'bursting' spontaneous firing rate, which made estimations of the frequency threshold response areas difficult.

All the units studied could be stimulated by the complex 'clicks' produced by finger-snapping or metallic contacts; sucking noises also proved very effective, especially for stimulating units with characteristic frequencies outside the human auditory range.

Seventeen units would respond only to these clicks, or to 'odd' sounds such as jangling keys. Most of them gave inconsistent responses unless the cat's attention was directed to the sound. Fourteen of these units were found in one cat which appeared to be less sensitive to sounds than the other cats in the series. In this animal, few units were found responding to tones, and their thresholds were comparatively high; her attention was difficult to attract by auditory stimuli.

Rather more than half of the units responding to tones showed, on stimulation, sustained excitation, or inhibition of their spontaneous firing. These units frequently had low spontaneous rates. Even at optimal stimulation by a tone, the firing was characteristically irregular in time and would slow during the course of a tone lasting a few seconds. In about a fifth of the units, this 'adaptation' was marked.

The majority of the remainder of the tone responders fired transiently, either at the onset or end of the tone, or at both points ('on-off').

Three units responded in more than one way to various tonal stimuli. Two of these exhibited on-off responses, with inhibition of the spontaneous firing during a sustained stimulus. In one of these the tone producing the on-off response was different from, and lower in frequency than, that producing the inhibition. The third unit displayed no off response, but gave an on response followed by inhibition to the same tone.

Classification of units studied

of 91 units (excluding 5 unresponsive units of 'abnormal shape')

70 responded to some acoustic stimulus (all responded to 'clicks')	78%
18 were unresponsive to any sound	20%
3 responded to movements in the visual field only	3%

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of the 70 'responsive' units

53 responded to tones	75%
10 responded to clicks only	15%
7 responded variably to 'odd' sounds	10%

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of 51 units responding to tones

21 showed sustained excitation	41%
6 showed sustained inhibition	12%
4 showed on-off responses only	8%
3 showed on responses only	6%
10 showed off responses only	20%
4 responded to gliding tones only	8%
3 showed mixed responses	6%

An important factor influencing the behaviour of the units was the extent of prior stimulation. All the units showed habituation to repeated stimuli to a greater or lesser extent, and in three it was most marked. It was often necessary to allow many seconds to elapse between each successive stimulus before reproducible frequency threshold curves could be obtained. One unit, which showed very slight habituation to brief stimuli, when stimulated continuously for two minutes at its characteristic frequency at 25 db above threshold, took over five minutes to recover to within 5 db of that threshold.

In one animal, three units were obtained responding not to sounds but to movements of a white coat in front of its head. This response was not obtained in the dark or when the cat closed its eyes. Although evidently visual, it could not be elicited by flashes of light or movement of a small light source. Stimulation of the face by stroking or of the cornea by puffs of air, was ineffective.

Frequency-Threshold Curves

About half of the units responding to tones did so at frequencies above 2o kc/s, 36 kc/s being the highest characteristic frequency observed. It is difficult to estimate how linear the output of the loud-speaker system is over the frequency ranges involved, but for the purpose of frequency-threshold measurements, the assumption of linearity has been made.

Frequency-threshold curves were obtained on 18 units. For each unit, the limits of the effective frequency band were determined at as many different intensities as possible. The test tones were of the order of one half to one second long; occasionally brief tones were more effective. Most of the 18 units were 'excitatory', and the majority of these responses were examined over a 40 db range, and one unit to 55 db above threshold.

The shapes of the curves obtained varied greatly from unit to unit. In general, the higher the characteristic frequency, the narrower the response curve, but considerable variation could be seen between units of similar frequencies. The units examined fell into two groups according to characteristic frequency, the first ranging from 1-5 kc/s, and the second from 25-35 kc/s. The bandwidths of the low frequency group at 15 db above threshold ranged from 0.5 to 1.6 octaves; those of the high frequency group from 0.1 to 0.5 octaves.

It was possible to examine the frequency response area of one low threshold unit over a 55 db intensity range. It was evident that at the highest intensity used the unit was not optimally stimulated, the bandwidth being reduced, compared with lower intensities, at the upper frequency limit, the unit actually exhibiting suppression at this point.

Responses to changing frequency

Four units were found which fired only when the stimulating frequency was changed over a small range, constant frequency tones being ineffective. Three were stimulated by 'swinging' the oscillator dial over a range of an octave or less. In the case of one unit, such 'swinging' produced an amplitude change of the order of $\pm$ 10%, and this may have been the stimulating factor. However, the rapid changes in intensity produced by tone onsets were ineffective.

Two of these units were subjected to various frequency-modulated tones from the F.M. generator. Only one responded to such stimulation, with single or multiple spikes occurring irregularly, but bearing a constant relationship to the phase of the frequency modulation. It did not respond to a steady tone. The position of firing, relative to the phase of modulation, changed as the modulating frequency changed. At a modulation frequency of 6 c/s, it fired as the oscillator frequency decreased, and at 10 c/s, as the frequency increased, the actual 'phase delay' being nearly 180°. It was not possible to determine the transmission time ('latency') to the onset of a steady tone, since the unit did not respond to this stimulus. However the minimum delay in firing after switching on the modulation was about 20 msec, a fairly 'normal' value for cortical latency. To account for the observed phase delay in terms of transmission time would require a latency of over 120 msec or six times as long. It does not seem therefore that the effect can be explained in this way and it seems more likely that some factor like rate of change of frequency may be responsible.

Of the units responding to tones, nine were subjected to F.M. tones. Where possible, a frequency-threshold curve was first determined, and then the unit 'stimulated' by F.M. sound with centre frequencies at various points about the curve. In this way, the phase of the resulting firing of the uhit to the F.M. modulation waveform could be compared with that expected from the position of the centre frequency of the F.M. tone to the frequency-response area. Six units responded with rhythmical firing of bursts which generally bore a constant phase relationship to the modulation waveform, this relationship being approximately as expected from the frequency response area. Thus each of five units stimulated with a centre frequency just below the lower limit of its frequency response area at the same intensity, responded with bursts of spikes as the frequency reached its maximum. In two units it was possible to stimulate with a centre frequency above the upper limit of the frequency-response area, and these units fired when the frequency was at a minimum. Most of the units responded by acceleration of their spontaneous rates, the dominant phase relationship being apparent in one, only by averaging out over several cycles of modulation; the acceleration in most units did not occur in every cycle.

Tonotopic organisation of units

The sites from which the units were recorded, have been determined in each case by noting the position of the micro-electrode with respect to the plug during recording, and relating the position of the latter to the cortex after its removal during perfusion of the brain at the end of the experiment.

Generally, the units were in one of three areas. The largest was bounded by the upper limits of the ecto-sylvian sulci, and showed an overall increase in characteristic frequency moving from the posterior ecto-sylvian sulcus to the anterior, and also showed an increase in characteristic frequency as one moved from the lateral to the medial limit, near the supra-sylvian sulcus. The second area lay bordering, and apparently in, the supra-sylvian sulcus, encroaching on the first area near the posterior ecto-sylvian sulcus. The units in this second area had characteristic frequencies, 25 kc/s and above. The third area lay anterior to the anterior ecto-sylvian sulcus, and contained units of characteristic frequency below 10 kc/s, several below 1 kc/s.

Detailed examination of these areas, however, showed no fine-scale progression of characteristic frequencies. Units of high frequency would be found posterior to those with low characteristic frequency; in the same puncture, units of widely differing frequencies would be found. In one such puncture, characteristic frequencies ranged from 4 kc/s to 30 kc/s with no correlation between frequency and depth. Nearer, and within the sulcus itself, it appeared that the deeper the units were situated, the lower the frequency, compared with units located more superficially.

No correlation between site and type of response could be found. Characteristically, units of widely differing response pattern would be obtained in the same puncture. At one site, two units were studied, one appearing after the other without moving the electrode. The first uhit exhibited sustained inhibition together with an on-off response, and had a wide frequency-response area, whilst the second was excited in a sustained manner by an identical characteristic frequency to the first, and had a narrow frequency-respohse curve. An 'off' unit, of the same characteristic frequency, was found 1.5 mm deeper in the same puncture.

Surface Electrocorticogram

No correlation could be seen by eye between the spikes recorded at the different depths and the overlying surface slow waves recorded by the indifferent gross electrode.

The E.C.G. reflected changes in the state of wakefulness of the cat, ranging from fast low voltage activity when fully awake, to slower, higher voltage activity strongly resembling the alpha rhythm, when drowsy and asleep. Slow waves could be easily evoked by clicks, and usually by tone onsets over a wide range of frequencies.

DISCUSSION

This technique of study of the neural unit responses of the unanaesthetised cortex has proved relatively simple and reliable. The main factor determining success or failure in obtaining data appears to be the state of the cortex at the time of recording; particularly, whether it is depressed by trauma, liquid paraffin, change of temperature or pressure. The E.C.G. is not necessarily a reliable guide to such depression, and cannot be used as an index when considering units which do not appear to respond to auditory stimuli, or do so unreliably, transiently, or with difficulty.

In spite of this problem, the unresponsive units studied in this series form a similar proportion of the total to the figures of Galambos (1960) from a similar series. This proportion is smaller than that found in the anaesthetised auditory cortex by Erulkar, Rose & Davies (1956).

The general behaviour of the units so far studied closely resembles that described by Galambos (1960). In particular, a higher proportion of units responding in a sustained manner to tones was found in the two studies, compared with the observations of Katsuki (1959b) in similar preparations. In view of the foregoing remarks on cortical condition it may be wondered whether this difference arises from the lack of 'damping' of cortical movements in Katsuki's series. Katsuki also found fewer units exhibiting narrow frequency response areas than in this study and that of Galambos.

Allanson & Whitfield (1955) described, in theory and experiment, a situation in which maximal tones might not be optimally effective in exciting units. One unit exhibiting such behaviour has been observed in this series. Galambos (1960), Hind (1960) and Erulkar, Rose & Davies (1956) also noted similar units.

Of the units studied, the overall picture of characteristic frequency versus position accords with the results of Galambos (1960) on the unanaesthetised, and Hind et al (1950) on the lightly anaesthetised cortex. They may also be reconciled in the main with the recent suggestion of cortical localisation of frequency in the cat, by Woolsey, (1960) which contrasts with Tunturi's work on the dog (e.g. summarised in 1960). The centre area described above would correspond to Woolsey's AI, and the area adjoining and apparently involving the supra-sylvian sulcus would correspond to his "Supra-sylvian fringe area". The low frequency area anterior to the anterior ecto-sylvian sulcus would correspond to the low frequency end of the AII area on his old classification (Woolsey &

Walzl 1942), or the low frequency end of the supra-sylvian fringe area on his new classification. However, the few units characterized in the present study in this last area appear to lie in a sequence opposite to that required by Woolsey, i.e. approaching the supra-sylvian sulcus, the characteristic frequencies of most of the units decreased. It is interesting that in this anterior area, the majority of the units observed showed transient responses, i.e. on, off, or on-off, only a small minority exhibiting sustained excitation or inhibition.

The finding of an apparent progression of characteristic frequencies towards higher values in a medial direction in the AI area, supports the results which Hind showed but did not discuss in his paper of 1953 (Hind 1953). Another progression appears to be one towards lower frequencies, as the supra-sylvian fissure area is penetrated more deeply. Obviously, further data from a larger sample of units spread over the AI and adjacent cortical areas need to be obtained before importance can be attached to these observations.

Of importance to this project is the finding of four units stimulated only by 'gliding' or frequency-modulated tones. Both directions of 'glide' appeared equally effective. In the visual system, Hubel (1959) has found some units more responsive to light movements in one direction than in the opposite direction.

Two of these units were subjected to frequency modulated tones, and responses obtained from one of them. It seems unlikely that the 'phase delay' exhibited by this unit on increasing the modulation frequency can be explained on the basis of transmission time from the periphery to the cortex.

Those units which responded to steady tonal stimuli showed, when stimulated by F.M. sound, firing patterns which agreed in phase with that expected from their steady state response areas. The lability of their firing resembles that observed in the evoked surface cortical waves described by Whitfield (1957).

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Implications for Future Work.

The work covered by this report has been largely of an exploratory nature.

Now that ways of controlling unwanted variables have been found, and a classification of the distribution of types of response carried out, it will be possible to study in more detail the firing patterns in relation to rate of change of stimulus. In particular it is proposed to study thoroughly those units which respond only to gliding tones, if sufficient of these can be located, in order to try to confirm, and if confirmed to elucidate, the interesting phase relations which have been observed. In view of the findings on the 'common' type of unit which gives a sustained response to a steady tone, it is proposed to use modulations of lower rates of change than heretofore to see whether these units can in fact be said to form a continuum with those responding only to glides.

Our present hypothesis suggests that amplitude and frequency changes should interact in a predictable way in respect of the behaviour of the total array of auditory fibres, and we propose also to study the effect of combined amplitude and frequency modulation on the response of single cortical units.

Personnel Utilized

Title	Number of Hours devoted to this contract	Cost
Graduate Investigator	Full-time	£1,100
Technical Assistant	Full-time	350
Director	1/8 full-time	300
Secretarial Assistant	1/5 full-time	100

Expendable supplies and materials	300
Overhead costs	400
Property acquired	Nil